#14 Declaration YV 6/12/01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Arntzen et al.

Application No.: 09/129,298 Group Art Unit: 1638

Filed: August 5, 1998 Examiner: O. Zaghmout

For: THE USE OF MIXED DUPLEX OLIGONUCLEOTIDES TO EFFECT LOCALIZED CHANGES IN PLANTS

Attorney Docket No.: 7991-023-999

DECLARATION OF DR. PETER R. BEETHAM UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, PETER R. BEETHAM, do declare and state that:

1. I am a resident of the United States, residing at 7128 Tanager Drive, Carlsbad, California, 92009.

2. I presently hold the position of Research Director in the Plant Industrial Products Division of ValiGen (US), Inc., the assignee of the present application, which position I have held since August 2000. I held the positions of Project Leader in the Plant Industrial Products Division of Kimeragen, Inc., the predecessor company of ValiGen (US), Inc., from January 2000 to August 2000; Senior Scientist at Kimeragen, Inc. from January 1999 to December 1999; post-doctoral fellow at Boyce Thompson Institute at Cornell University from 1997 to 1999; and Scientific Officer SCI-2 in the Victorian Department of Agriculture and Rural Affairs in Australia from 1985-1992. Attached as Exhibit 1 is a copy of my *Curriculum vitae*.

3. I received the degree of Doctor of Philosophy from Queensland University of Technology, Brisbane, Australia in 1999; and a BSc (Hons) from Monash University, Victoria, Australia in 1984.

4. I have read and am familiar with the specification of the above-identified application. I have been informed that claims of the above-identified patent application relating to methods of making a localized mutation causing a desired trait in a target gene in a plant cell comprising adhering to a particle a recombinagenic oligonucleobase, introducing the particle into a cell of a population of plant cells and identifying a cell of the population having a mutation, or comprising perforating the cell walls of a population of plant cells, introducing a recombinagenic oligonucleobase and identifying a cell of the population having a mutation, are subject to a rejection based on obviousness because it is alleged that the teachings of U.S. Patent Nos. 5,565,350; 5,731,181; and 5,204,253 render obvious the claimed methods to one skilled in the art.

5. I am a scientific investigator and manager of technical research and development. My professional research interests have focused upon a number of areas including plant molecular biology, plant molecular virology, plant viral gene promoters, plant transformation technologies, and gene targeting in plant systems. My scientific research has focused on the physiology and molecular biology of plants and plant viruses for the last fifteen years. I have been involved in plant tissue culture, plant genetic engineering, and more recently commercial applications for plant biotechnology.

6. My research experience also includes the use of biolistics technology, the technology described in U.S. Patent No. 5,204,253. More than one thousand uses of biolistics technology in plant systems have been performed either by me personally or under my supervision and control. As a consequence of my own experience and knowledge of the scientific literature concerning plant molecular biotechnology, I am very familiar with the technical capabilities of those who routinely perform research in the fields of plant genetics, plant molecular biology, and plant transformation.

7. The biolistics technology taught by U.S. Patent No. 5,204,253 and in the present specification is a technology used, *inter alia*, to transfer nucleic acid molecules into a cell. The technology requires that the nucleic acid molecules be precipitated onto microparticle projectiles, usually a suspension of gold particles one micron in diameter, in a harsh solution of salts, *e.g.*, calcium chloride, and positively charged proteins, *e.g.*, spermadine. The nucleic acid-coated particles are then literally "shot" into cells. The application of biolistics technology to the delivery of nucleotide constructs in plants has generally been limited to double-stranded DNA plasmids typically larger than two kilobases in length, often five or more kilobases in length. See, *e.g.*, Gene Transfer To Plants, 1995, Potrykus and Spangenberg, Eds., Chapters 14-20, Springer Lab Manual, Springer Verlag, pp. 115-169, attached as Exhibit 2; and Sanford et al., 1993, Methods in Enzymology 217:483-509, attached as Exhibit 3. At the time the present invention was made, it was common knowledge among the scientists who were performing plant biolistics that the nucleotide constructs used in this process should be supercoiled. Nicked and linearized structures were known to reduce efficiency. See, *e.g.*, Gene Transfer To Plants, 1995, Potrykus and Spangenberg, Eds., Chapters 14-20, Springer Lab Manual, Springer Verlag, pp. 115-169. In fact, during my doctoral studies I analyzed plasmid preparations of different quality, *i.e.*, supercoiled, nicked, linear. The different preparations were each adhered to biolistics particles and introduced into a population of plant cells. The results showed that higher quality plasmid preparations (mainly supercoiled) had higher levels of transformation than lesser quality plasmid preparations (mainly nicked and/or linear), as measured by detecting expression of a marker gene encoded by the plasmid. Although linear nucleic acid molecules have been transformed into plant cells using biolistics technology, *e.g.*, plant virus genomes, these nucleic acid molecules were always greater than a kilobase, more routinely greater than five kilobases. See, *e.g.*, Wang et al. 2000, Virus Genes 20:11-17, attached as Exhibit 4.

8. In complete contrast, the recombinagenic oligonucleobases of the present invention are radically different in size and structure than the nucleic acid molecules that were typically employed and known to work in biolistics technology applications at the time the present invention was made. The recombinagenic oligonucleobases of the present invention are as much as two orders of magnitude smaller, and are linearized, single-stranded

structures with regions of secondary structure. Moreover, the secondary structure of the oligonucleobase is required for activity. The precise mechanism by which the recombinagenic oligonucleobase molecules precipitate onto the gold particles and are subsequently resolubilized after delivery of the particles into plant cells is not well characterized.

9. Based on the foregoing, it is my opinion, and I believe that a scientist knowledgeable in the field of plant molecular biology and plant transformation would also hold the opinion, that the cited prior art references do not provide the required reasonable expectation of success in achieving the claimed methods because it could not have been reasonably predicted that the recombinagenic oligonucleobases could be successfully adhered to the biolistics particle and resolubilized off the particle once in the plant cell, and that it could not have been reasonably predicted that the required secondary structure of the oligonucleobase molecule would be maintained throughout the biolistics method for successfully making a desired localized mutation in a target gene.

10. Based on my research experience and the foregoing discussion, I conclude, and I believe a scientist knowledgeable in the field of plant molecular biology and plant transformation would also conclude, that the teachings of U.S. Patent Nos. 5,565,350; 5,731,181; and 5,204,253, either alone or in combination, do not render obvious methods of making a localized mutation causing a desired trait in a target gene in a plant cell comprising adhering to a particle a recombinagenic oligonucleobase, introducing the particle into a cell of a population of plant cells and identifying a cell of the population having a mutation, or comprising perforating the cell walls of a population of plant cells, introducing a recombinagenic oligonucleobase and identifying a cell of the population having a mutation, because the prior art does not provide a reasonable expectation of success in achieving such methods since recombinagenic oligonucleobases are sufficiently different from the double-stranded and single-stranded nucleic acid molecules employed in prior art biolistics methods. Accordingly, I conclude, and I believe a scientist knowledgeable in the field of plant molecular biology and plant transformation would also conclude, that the claimed methods of the present invention are nonobvious in view of the cited prior art.

11. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the specification or any patent issuing thereon.

Dated: 6/6/2001

Peter R. Beetham, Ph.D.

Attachments:

Exhibit 1: *Curriculum vitae*

Exhibit 2: Gene Transfer To Plants, 1995, Potrykus and Spangenberg, Eds., Chapters 14-20, Springer Lab Manual, Springer Verlag, pp. 115-169

Exhibit 3: Sanford et al., 1993, Methods in Enzymology 217:483-509

Exhibit 4: Wang et al. 2000, Virus Genes 20:11-17